No Inhibition of Cytochrome P450 Activities in Human Liver Microsomes by Sulpiride, an Antipsychotic Drug

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The effects of sulpiride, an antipsychotic drug, on cytochrome P450 (CYP) activities in human liver microsomes were investigated. Sulpiride at 50 or 500 μM concentration neither inhibited nor stimulated CYP1A2-mediated 7-ethoxyresorufin *O*-deethylation, CYP2C9-mediated tolbutamide hydroxylation, CYP2C19-mediated *S*-mephenytoin 4′-hydroxylation, CYP2D6-mediated debrisoquine 4-hydroxylation, CYP2E1-mediated chlorzoxazone 6-hydroxylation, CYP3A4-mediated nifedipine oxidation, or CYP3A4-mediated testosterone 6β-hydroxylation. The free fractions of sulpiride in the incubation mixture estimated by ultracentrifugation were more than 90.5%. These results suggest that sulpiride would not cause clinically significant interactions with other drugs, which are metabolized by CYPs, *via* the inhibition of metabolism.

Key words   sulpiride; antipsychotic drug; human liver microsomes; cytochrome P450; drug interaction

Cytochrome P450s (CYP) comprise a superfamily of enzymes that catalyze the oxidation of a wide variety of xenobiotic chemicals, including drugs and carcinogens.[1—3] Multiple drug therapy is a common therapeutic practice, particularly in patients with several diseases or conditions, and many drug–drug interactions involving metabolic inhibition are being reported.[4,5]

Sulpiride, an oral selective antagonist of the dopamine D_2 receptor, is widely used for the treatment of schizophrenia, depression, and gastric and duodenal ulcers.[6,7] After oral administration of 100—400 mg in humans, peak plasma levels of 0.2—1.5 μg/ml are obtained within 1—3 h, and the elimination half life is 7—8 h.[8—10] After intravenous administration of sulpiride, 70% of the dose is recovered as unchanged drug in urine, as compared to 15% after oral administration,[11] and the bioavailability of the oral form is 25—36%.[10,11] After oral dosing of ^{14}C-labeled sulpiride, urinary excretion of radioactivity was 27—52%, and more than 95% of the radioactivity recovered in the urine and feces is unchanged sulpiride.[8] It has been reported that sulpiride is co-administered with other drugs including olanzapine,[12,13] fluvoxamine,[14] and cimetidine[15] for the treatment of schizophrenia, depression, and gastro-duodenal ulcers, respectively. However, there are few studies on the effect of sulpiride on human hepatic CYP-mediated drug-metabolizing activity, nor have there been any *in vivo* pharmacokinetic interaction studies.

Recent studies have shown that inhibition constants (K_i) for drugs as inhibitors of microsomal drug-metabolizing enzymes, such as CYP, should be correlated in conjunction with the extent of nonspecific binding to components of the *in vitro* matrix by measurement of the unbound fraction under the incubation condition to yield more accurate determination of the constant.[16—18]

In the present study, we investigated the effects of sulpiride on specific activities by CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in human liver microsomes, and the results suggest that sulpiride is not an inhibitor of these CYPs.

MATERIALS AND METHODS

Materials   Pooled human liver microsomes from 46 individuals were obtained from XenoTech (Lenexa, KS, U.S.A.). Sulpiride, 7-ethoxyresorufin, resorufin, tolbutamide, debrisoquine sulfate, chlorzoxazone, nifedipine, sulfaphenazole, tranylcypromine, quinidine hydrochloride, and diethyldicarbamate were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). *S*-Mephenytoin, 4′-hydroxymephenytoin, hydroxytolbutamide, 6-hydroxychlorzoxazone, oxidized nifedipine, and 6β-hydroxytestosterone were obtained from Ultrafine Chemicals (Manchester, U.K.). Testosterone, phenobarbital sodium, and *p*-hydroxybenzoic acid isopropyl were purchased from Nacalai Tesque (Kyoto, Japan), Wako Pure Chemicals (Osaka, Japan), and Tokyo Chemical Industry (Tokyo, Japan), respectively. All other reagents were of the highest purity commercially available.

Determination of Human CYP Activities   7-Ethoxyresorufin *O*-deethylase activity (CYP1A2), debrisoquine 4-hydroxylase activity (CYP2D6), and nifedipine oxidase activity (CYP3A4) in the presence or absence of sulpiride were determined as described previously.[19] The incubation mixture consisted of 0.05—0.5 mg/ml of human microsomes, 2 mM NADP+, 10 mM glucose-6-phosphate, 5 mM magnesium chloride, 1 unit/ml of glucose-6-phosphate dehydrogenase, 100 mM phosphate buffer (pH 7.4), and 5 μl of methanol or 5—50 mM sulpiride dissolved in methanol in a final volume of 500 μl.[19] The microsomal protein concentration in the mixture was 0.05 (nifedipine oxidation), 0.1 (for 7-ethoxyresorufin *O*-deethylation and testosterone 6β-hydroxylation), 0.2 (for chlorzoxazone 6-hydroxylation), or 0.5 mg/ml (for tolbutamide hydroxylation, *S*-mephenytoin 4′-hydroxylation, and debrisoquine 4-hydroxylation). Because the K_m for 7-ethoxyresorufin *O*-deethylation, tolbutamide hydroxylation, *S*-mephenytoin 4′-hydroxylation, debrisoquine 4-hydroxylation, chlorzoxazone 6-hydroxylation, nifedipine oxidation, and testosterone 6β-hydroxylation by human liver microsomes were 0.22, 150.8, 27.3, 83.9, 47.7, 12.2, and 50.3 μM, respectively (data not shown),[19] concentrations of 7-ethoxy-

resorufin, tolbutamide, *S*-mephenytoin, debrisoquine, chlorzoxazone, nifedipine, and testosterone were 0.25, 200, 30, 100, 50, 10, and 50 μM, respectively, which are around the expected K_m. Incubation was carried out at 37 °C for 5 min (for testosterone 6β-hydroxylation), 10 min (for 7-ethoxyresorufin *O*-deethylation, chlorzoxazone 6-hydroxylation, and nifedipine oxidation), 30 min (for tolbutamide hydroxylation and *S*-mephenytoin 4′-hydroxylation), or 60 min (for debrisoquine 4-hydroxylation).

For the assay of hydroxytolbutamide, the reaction was terminated by the addition of 100 μl of 1 M hydrochloric acid. After 50 μl of 20 μg/ml phenobarbital sodium, an internal standard and 3 ml of ethyl acetate/hexane (1 : 1) was added, then the mixture was shaken for 10 min and centrifuged at 1900 g for 5 min. The organic phase (2.5 ml) was evaporated under nitrogen. The residue was dissolved immediately in 200 μl HPLC mobile phase (Eluent A) and 80 μl was injected onto an HPLC column. The HPLC system consisted of a Waters model 600S System Controller, Waters model 717 Autosampler, Waters model 486 Tunable Absorbance Detector, and an analytical column Inertsil ODS-3 (150×4.6 mm I.D., GL Sciences Inc., Tokyo, Japan) equipped with a TSKguardgel ODS-80Ts cartridge (3.2× 15 mm, Tosoh Co., Tokyo, Japan). The column temperature was set at 40 °C, and a UV-detector was set at 240 nm. The mobile phase was 5 mM potassium dihydrogen phosphate/methanol (58 : 42) as eluent A and 5 mM potassium dihydrogen phosphate/methanol (20 : 80) as eluent B. Gradient conditions were 0—12 min, 0% B; 12—12.1 min, 0—100% B (linear gradient); 12.1—20 min, 100% B; 20—20.1 min, 100—0% B (linear gradient); 20.1—28 min 0% B. Flow rate was held at 1 ml/min for 12 min and increased to 1.4 ml/min at 12.1 min. Calibration curves were linear for hydroxytolbutamide concentrations from 0.2 to 10 μM.

For the assay of 4′-hydroxymephenytoin, the reaction was terminated by the addition of 100 μl of 1 M hydrochloric acid. After 100 μl of 2 μg/ml phenobarbital sodium, an internal standard, and 3 ml of ethyl acetate/hexane (1 : 1) was added, the mixture was shaken for 10 min and centrifuged at 1900 g for 5 min. The organic phase (2.5 ml) was evaporated under nitrogen. The residue was dissolved immediately in 200 μl HPLC mobile phase (Eluent A), and 80 μl was injected onto an HPLC column. The same HPLC system and column as described above were used, except that the UV-detector was set at 204 nm. The column temperature was set at 45 °C. The mobile phase was 5 mM potassium dihydrogen phosphate/methanol (70 : 30) as eluent A, and 5 mM potassium dihydrogen phosphate/methanol (20 : 80) as eluent B. Gradient conditions were 0—21 min, 0% B; 21—21.1 min, 0—100% B (linear gradient); 21.1—30 min, 100% B; 30—30.1 min, 100—0% B (linear gradient); 30.1—38 min 0% B. Flow rate was held at 1 ml/min for 23 min and increased to 1.4 ml/min at 23.1 min. Calibration curves were linear for 4′-hydroxymephenytoin concentrations from 0.1 to 5 μM.

For the assay of 6-hydroxychlorzoxazone, the reaction was terminated by the addition of 25 μl of 85% phosphoric acid. After 50 μl of 20 μg/ml phenobarbital sodium, an internal standard, and 3 ml of ethyl acetate/hexane (1 : 1) was added, the mixture was shaken for 10 min and centrifuged at 1900 g for 5 min. The organic phase (2.5 ml) was evaporated under nitrogen. The residue was dissolved immediately in 200 μl

HPLC mobile phase (Eluent A), and 80 μl was injected onto an HPLC column. The same HPLC system and column as described above were used except that the UV-detector was set at 240 nm. The column temperature was set at 40 °C. The mobile phase was 20 mM sodium perchlorate buffer (pH 2.5)/methanol (68 : 32) as eluent A and 20 mM sodium perchlorate buffer (pH 2.5)/methanol (20 : 80) as eluent B. Gradient conditions were 0—18 min, 0% B; 18—18.1 min, 0—100% B (linear gradient); 18.1—25 min, 100% B; 25—25.1 min, 100—0% B (linear gradient); 25.1—35 min 0% B. Flow rate was held at 1 ml/min for 10 min and increased to 1.4 ml/min at 10.1 min. Calibration curves were linear for 4′-hydroxymephenytoin concentrations from 0.2 to 5 μM.

For the assay of 6β-hydroxytestosterone, the reaction was terminated by the addition of 3.5 ml of ethyl acetate, and the mixture was shaken for 10 min and centrifuged at 1900 g for 5 min. The organic phase (3 ml) was evaporated under nitrogen. The residue was dissolved immediately in 200 μl of 50% methanol, and 80 μl was injected onto an HPLC column. The same HPLC system as described above was used, except that the UV-detector was set at 239 nm. An analytical column was Inertsil ODS-2 (250×4.6 mm I.D., GL Sciences Inc.) equipped with a TSKguardgel ODS-80Ts cartridge (3.2×15 mm), and the column temperature was set at 50 °C. The mobile phase was water/methanol (4 : 1) as eluent A and water/methanol (1 : 10) as eluent B. Gradient conditions were 0—1 min, 35% B; 1—18 min, 35—38% B (linear gradient); 18—18.1 min, 38—100% B (linear gradient); 18.1—22 min, 100% B; 22—22.1 min, 100—35% B (linear gradient); 22.1—25.5 min 35% B. Flow rate was held at 1 ml/min for 20 min and increased to 1.3 ml/min at 20.1 min. Calibration curves were linear for 4′-hydroxymephenytoin concentrations from 0.1 to 10 μM.

For the positive control, 5 μl of 2 mM furafylline dissolved in methanol (CYP1A2),[20—22] 2 mM sulfaphenazole in 50% acetone (CYP2C9),[21—24] 2.5 mM tranylcypromine in 50% acetone (CYP2C19),[22] 100 μM quinidine hydrochloride in water (CYP2D6),[21,22,25] or 50 μM ketoconazole in methanol (CYP3A4),[21,23] or 50 μl of 1 mM diethyldithiocarbamate in water (CYP2E1)[21,22] was added instead of sulpiride.

Determination of Free Fraction in Incubation Mixture The incubation mixture consisted of human microsomes (0.05—0.5 mg/ml), 50 or 500 μM sulpiride, and 100 mM phosphate buffer (pH 7.4) in a final volume of 500 μl. After a 5-min incubation at 37 °C, the mixture was centrifuged at 105000 g for 60 min at 25 °C,[17,18,26] and the concentration of sulpiride in the supernatant was measured by HPLC with an analytical column, Inertsil ODS-3 (150×4.6 mm I.D.). The column temperature was set at 40 °C. The elution was conducted with 10% acetonitrile in 20 mM potassium phosphate buffer (pH 3.5) containing 0.1% triethylamine at a flow rate of 1 ml/min, and detection was by UV absorbance at 288 nm. Calibration curves were linear for sulpiride concentrations from 10 to 1000 μM.

RESULTS AND DISCUSSION

The inhibitory effects of sulpiride on metabolic activities in human hepatic microsomes are summarized in Table 1. 7-Ethoxyresorufin *O*-deethylase, tolbutamide hydroxylase, *S*-mephenytoin 4′-hydroxylase, debrisoquine 4-hydroxylase,

Table 1. Effect of Sulpiride on Metabolic Activities in Human Liver Microsomes

CYP	Reaction	Sulpiride (μM):	Metabolic activity (pmol/min/mg)			Positive control	Metabolic activity (pmol/min/mg)	
			0 (Control)	50	500		− (Control)	+
CYP1A2	7-Ethoxyresorufin O-deethylation		9.81 (100)	10.53 (107.3)	9.81 (100.0)	Furafylline 20 μM	9.78 (100)	3.38 (34.6)
CYP2C9	Tolbutamide hydroxylation		140 (100)	132 (94.2)	120 (86.0)	Sulfaphenazole 20 μM	175 (100)	28 (16.0)
CYP2C19	S-Mephenytoin 4′-hydroxylation		37.7 (100)	39.0 (103.4)	38.7 (102.6)	Tranylcypromine 25 μM	38.8 (100)	<6.7 (<17.2)
CYP2D6	Debrisoquine 4-hydroxylation		23.3 (100)	23.5 (100.7)	22.0 (94.4)	Quinidine 1 μM	26.6 (100)	9.2 (34.5)
CYP2E1	Chlorzoxazone 6-hydroxylation		881 (100)	803 (91.1)	842 (95.6)	Diethyldithiocarbamate 100 μM	1234 (100)	248 (20.1)
CYP3A4	Nifedipine oxidation		605 (100)	617 (102.0)	570 (94.2)	Ketoconazole 0.5 μM	624 (100)	<200 (<32.1)
CYP3A4	Testosterone 6β-hydroxylation		2501 (100)	2663 (106.5)	2492 (99.6)	Ketoconazole 0.5 μM	2643 (100)	<200 (<7.6)

Results represent means of duplicate determinations. Values in parentheses show percentage of control.

chlorzoxazone 6-hydroxylase, nifedipine oxidase, and testosterone 6β-hydroxylase activities in the presence of sulpiride at a concentration of 50 or 500 μM were 86.0—107.3% of the control, indicating that sulpiride had neither inhibitory nor stimulatory effects. On the other hand, the enzyme activities decreased to less than 34.6% of the respective control activities by inhibitors of the positive control.

The free fractions of sulpiride in the incubation mixture (0.05—0.5 mg protein/ml) were determined at concentrations of 50 and 500 μM. The free fractions of sulpiride estimated by ultracentrifugation were more than 90.5%, and the values were constant through the protein concentrations (Table 2). These results indicate that the protein binding of sulpiride in the incubation mixture is of minor importance.

When the substrate concentration is much lower than the K_m value, the degree of inhibition (R) can be expressed by the following equation, independent of the inhibition type, except in the case of uncompetitive inhibition[27,28]:

$$R = 1/(1 + I_u/K_i)$$

where I_u is the unbound concentration of the inhibitor. Additionally, when the absorption rate is maximum, the maximum inflow concentration of the inhibitor into liver ($I_{in,max}$) can be expressed as:

$$I_{in,max} = I_{p,max} \cdot R_B + \{(k_a \cdot D/Q_H) \cdot F_a\}$$

where $I_{p,max}$, R_B, k_a, D, Q_H, and F_a represent the maximum plasma concentration of the inhibitor in the circulation, blood-to-plasma concentration ratio, absorption rate constant, dose, hepatic blood flow, and the fraction absorbed from the gastrointestinal tract into the portal vein, respectively. After an oral dosing of 400 mg sulpiride in healthy volunteers, the peak plasma concentration ($I_{p,max}$) was 1.468 μg/ml (4.3 μM).[10] Protein binding of sulpiride in human plasma, which is measured by equilibrium dialysis, is 11—18% (mean; 14%).[29] Free fraction of $I_{in,max}$ for sulpiride after an oral dosing of 400 mg is calculated, using free fraction in plasma (f_u)=0.86, R_B=1 (R_B was assumed to be 1 because R_B has not been reported), k_a=0.1 min^{-1}, Q_H=1610 ml/min, F_a=1 to avoid false-negative predictions,

Table 2. Free Fraction of Sulpiride in Reaction Mixture Containing Human Liver Microsomes

Protein concentration in reaction mixture (mg/ml)	Sulpiride (μM):	Free fraction (%)	
		50	500
0.05		95.9	100.0
0.1		100.0	90.5
0.2		95.8	95.0
0.5		94.0	96.2

Results represent means of duplicate determinations.

to be 66 μM. In this paper, we have demonstrated that the inhibition of human CYPs by sulpiride was not observed at 500 μM concentration (Table 1), which is 8-fold higher than the predicted free fractions of $I_{in,max}$. Therefore, it is speculated that the K_i values would be much higher than the predicted free fractions of $I_{in,max}$, even if sulpiride inhibited human CYPs, and the results suggest that sulpiride might not affect the pharmacokinetics or metabolism of drugs metabolized by CYPs. Additionally, there are no clinical reports that sulpiride increases the blood concentrations of other CYP-metabolized drugs as a result of its inhibition of metabolism.

In conclusion, the present study demonstrated that sulpiride at 500 μM concentration, which is 8-fold higher than the predicted free fractions of $I_{in,max}$, did not inhibit human CYPs at all, suggesting that sulpiride would not cause clinically significant interactions with other drugs, which are metabolized by CYPs, via the inhibition of metabolism.

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